What is SeaKleen and How Does it Work?
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A very rare number of trees produce antibiotic-medicinals, among these being the tropical *Combretum molle*, which makes mollic and imberic acid, both having anti *Schistosoma* activity without being harmful to mammals. The Black Walnut, *Juglans nigra* and *J. regia*, produce quinones, among these being juglone and menadione (vitamin K3). However, menadione is ubiquitous throughout the plant and animal kingdom and it is used medicinally to stop bleeding. It also occurs in the human gut where it is produced by bacteria and without its production hemophilia would be the norm. In the US, hospitals use vitamin K in new born infants in order to accommodate its delayed production by intestinal bacteria. Additionally, it has certain positive attributes in controlling pests in aqueous systems.

Because of the economics, menadione (SeaKleen®) became the focus of research attention to control pests in ships' ballast water and it was subsequently patented for this use (US Patent #6,340,468) The material has the characteristics of being effective, economical, user friendly, and environmentally safe. Among the organisms controlled were Phytoplankton species, toxic dinoflagellates, dinoflagellates cysts, Zebra mussel larvae, Sheepshead minnow eggs and larvae, Fathead minnow larvae, Mysid shrimp larvae, Grass shrimp larvae, Copepods, Spiny water flea, benthic amphipod protozoans and bacteria (*E. coli* and *Vibrio fisheri*).

The possible negative effects on protected species did not go unnoticed and, therefore, independent tests were conducted on the sensitive economic species, *Mytilus galloprovincialis*. Essentially, results with menadione, or menadione plus menadione sodium bisulfite, showed that there was a sharp cutoff in toxicity at 0.2 ppm. These assays were conducted under light and dark conditions because of the effects of light on menadione, which is destroyed by light when in solution. This study unequivocally demonstrated that when diluted below a certain threshold the compound has no deleterious environmental effects. Tests against nuisance species showed that in *Isochrysis galbana* 1.5 and 0.75 ppm totally killed the organism within 24 hours. Similarly, *Glenodinium foliaceum* was annihilated within 6 hours with 1.5 ppm SeaKleen® and cysts were blitzed following 2 hours exposure to 2.0 ppm SeaKleen®. However, in these studies, doses of 0.25 ppm had little to no effect on the test organism.

It became evident, as a result of other independent studies, that a 1 to 2 fold dilution causes a loss in toxicity of SeaKleen® and that release from a ship has a multiple dilution effect which is proportional to the distance from the ship.

Further evaluations, conducted in New Zealand, showed the breakdown of SeaKleen® in fresh water and sea water under light and dark conditions, <u>without</u> any organisms. In river water, under sunlight, only 8% of the SeaKleen® was present at 72 hours. In seawater, exposed to sunlight, 47% of the SeaKleen® was present after 72 hours. In darkness, river water had 22% residual SeaKleen® after 28 days, while in seawater, 21% of the SeaKleen® remained. However, and most importantly, further studies showed that in river water exposed to Blue-Green Algae, SeaKleen® was below detectable limits (by HPLC) of 0.56 ppb at 48 hours. Hence, the presence of organisms directly affects the breakdown of SeaKleen® because at least one molecule of the material is used up by each organism.

The mode of action of SeaKleen® is multifaceted. First is the flow of electrons that results in the release of singlet oxygen, a very active species of oxygen. Although all living organisms require this active form of oxygen, its production is in limited quantities. In amounts slightly higher than normal, singlet oxygen is a potent agent which destroys pigments (i.e., chlorophyll) and causes peroxidation of membrane lipids, thereby destroying the architecture of the cell. This reaction can be controlled through use of appropriate doses of menadione. Also, due to its physiological role, singlet oxygen has the added advantage of being very short lived.

Menadione has been used for over 50 years in chicken and turkey feed, swine, cattle (to control dicoumarol poisoning), duck, pheasant, geese and fish (salmon and catfish). The material is used in catfish farming at the rate of 4.4 ppm, in the meal, where it is liberally spread into water; 3,193 Kg of menadione are used annually in the aquaculture of catfish. Menadione is also used in K-VIT hand cream at a concentration of 1%. Although it has been used for over 50 years, we have not been able to find any deleterious effects from these uses of menadione.